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NEW PLANKTON SAMPLERS

by

Sigeru Motoda

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I. Vertical successive plankton sampler (Figs. 1–4)

In studying the vertical distribution of plankton in the deep sea the collection is usually made by the aid of closing mechanism of the net. This method, however, requires much time, for the collection must be repeated from several different depths. The writer has devised a new sampler which is able to collect the plankton population separately from several different layers (five layers) by a single vertical haul.

The cylindrical apparatus, 15.5 cm in diameter and 35.5 cm in total height, is attached at the terminal end of the ordinary plankton net, instead of a simple bucket. The net used is 57 cm in mouth diameter, 200 cm in length and made of bolting cloth, 42.5 meshes per inch in upper 120 cm and 56.5 meshes per inch in lower 80 cm.

During the haul upward, the propeller revolved by water current operates to revolve the supporting disc, which bears five small store-nets (3.5 cm in diameter, 5 cm in length, made of bolting cloth 56.5 meshes per inch) arranged in a circle. As the connection between the upper large condensing net and underlying small nets is made at an eccentric hole of the disc, the small nets connected with the upper large net are succesively alternated with revolution of the supporting disc. Thus, the plankton organisms sampled at different depths are stored in the different small nets. When the disc accomplishes one round, a rod hanging on the margin of the disc slips down through the marginal slit of the disc, and stops the propeller, no more sampling being made.

The vertical distance of sampling can be regulated for three grades by changing the gear ratio (1/508.76, 1/1014.4 and 1/2051.27). The largest distance is kept as about 1200 metres for five layers (about 240 metres for one layer), the moderate distance is kept as about 600 metres for five layers (about 120 metres for one layer), . and the smallest distance is kept as about 300 metres for five layers (about 60 metres for one layer).

This apparatus was registered as patent No. 192461 of Tsurumi Seiki-Kosakusho at Tsurumi, Yokohama, on January 25th 1952.

II. High-speed successive plankton sampler (Figs. 5–7)

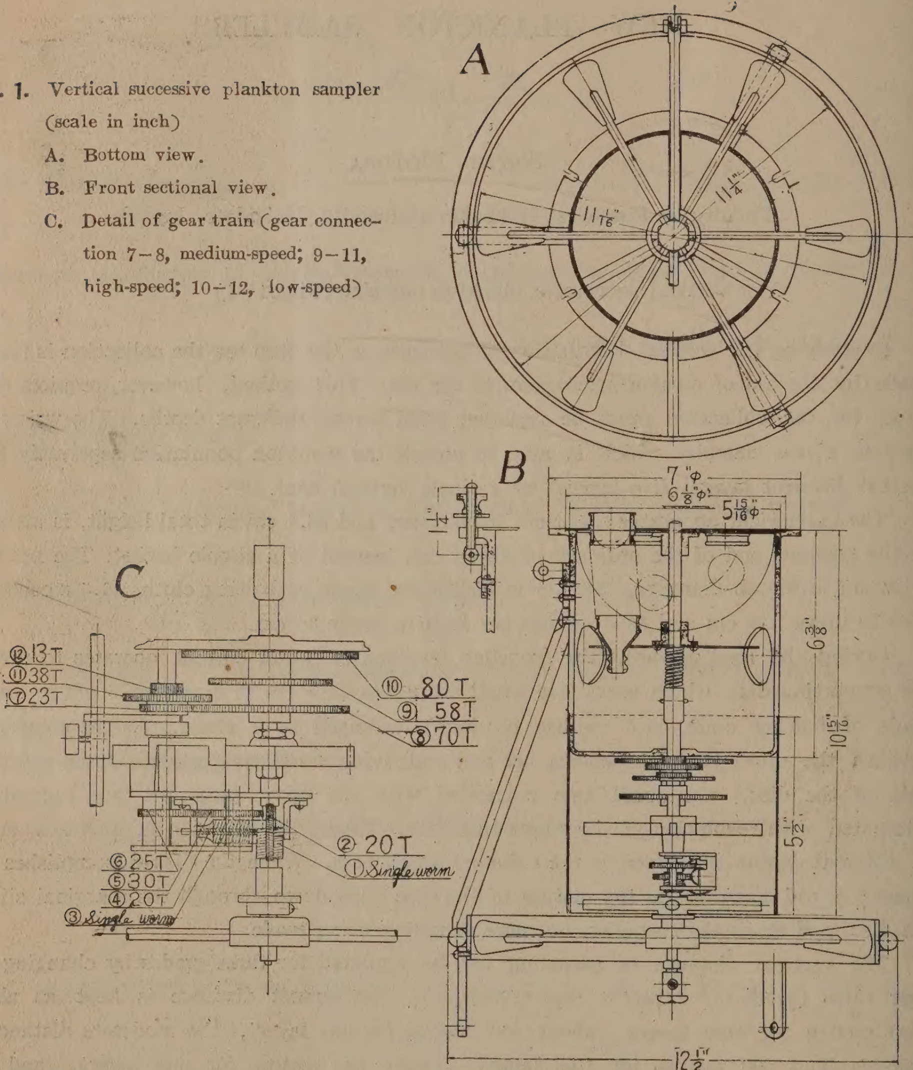
Since Hensen devised the "basket net" in 1888, several different patterns of under-way sampling apparatuses have been developed; there are Borgert's tow net (Steuer: Plank-

Fig. 1. Vertical successive plankton sampler (scale in inch)

A. Bottom view.

B. Front sectional view.

C. Detail of gear train (gear connection 7–8, medium-speed; 9–11, high-speed; 10–12, low-speed)



tonkunde), Monti's collector (Monti, 1910–11), Hardy's "plankton indicator" (Hardy, 1926a, 1926b, 1934, 1936a, Henderson et al., 1936), Hardy's "continuous plankton recorder" (Hardy, 1935, 1936b), Tamura's collector (Tamura, 1948), Tester and Stevenson's "metal net" (Tester and Stevenson, 1949), "high-speed plankton collector" of the California sardine research group (Marine Research Committee, 1950) and Gauld and Beganal's tow net (Gauld and Beganal, 1951).

Most of them are comparatively simple apparatuses, but a few are somewhat

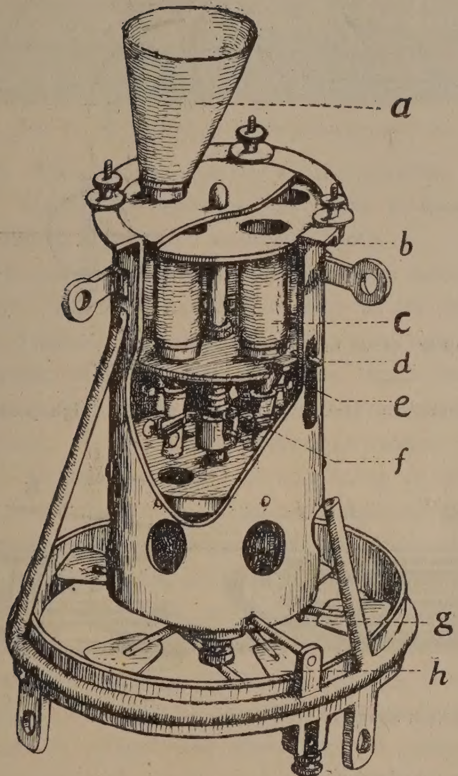


Fig. 2. Sketch of vertical successive plankton sampler.

a. Condensing net.

b. Upper revolving disc which bears store-nets.

c. Store-net.

d. Upper bending end of the stopper.

e. Marginal slit of the lower revolving disc.

f. Spring which forces the revolving disc upwards.

g. Terminal end of the stopper.

h. Propeller.

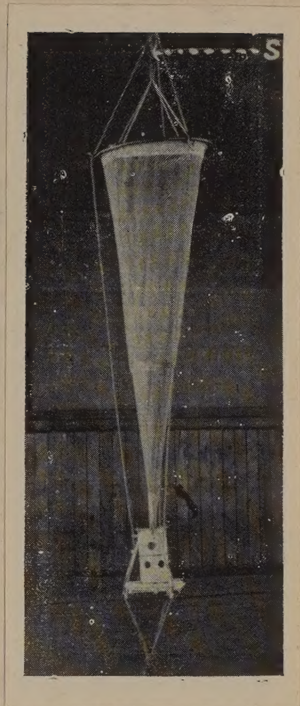


Fig. 3. Vertical successive plankton sampler, s: Swivel.

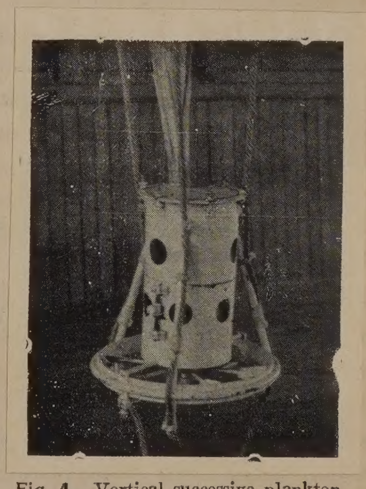


Fig. 4. Vertical successive plankton sampler.

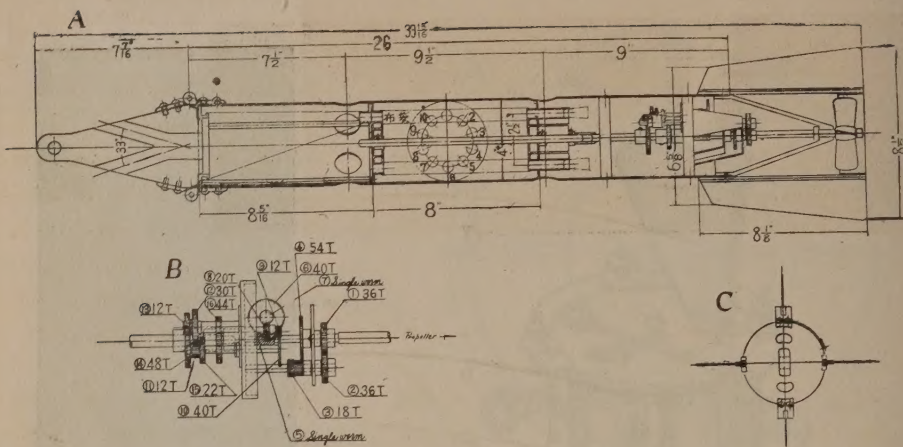


Fig. 5. High-speed successive plankton sampler (scale in inch).

A. Sectional view.

B. Detail of gear train (gear connection 11−12, low-speed; 15−16, high-speed).

C. Rear view.

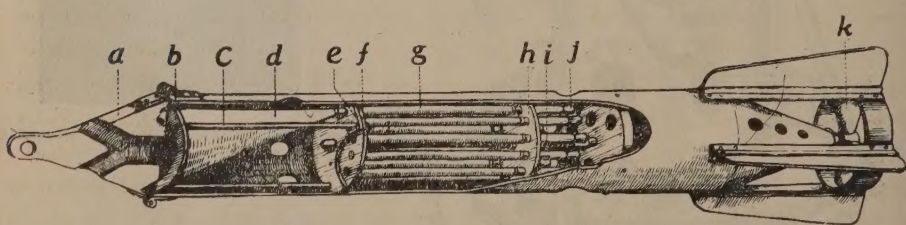


Fig. 6. Sketch of high-speed successive plankton sampler.

a. Solid head piece.

b. Anterior fixed disc which bears condensing net.

c. Supporting rod between anterior and posterior fixed disc.

d. Condensing net.

e. Posterior fixed disc.

f. Anterior revolving disc which bears store-nets.

g. Store-net.

h. Posterior revolving disc.

i. Spring which forces the revolving disc forwards.

j. Rubber plug.

k. Propeller.

complicated in their structure. The "continuous plankton recorder" is equipped with rolling mechanisms of the bolting cloth and is fit to provide quantitative and qualitative samples over wide areas. The "high-speed plankton collector" is equipped with depth-flow meter to know the exact depth at which the collection is made and the quantity of water

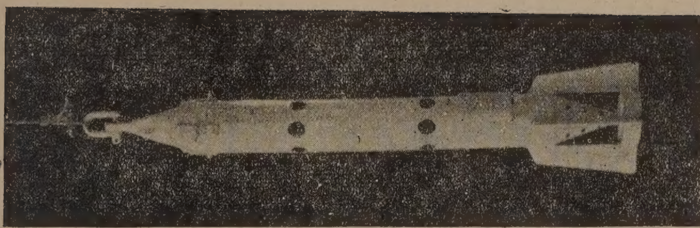


Fig. 7. High-speed successive plankton sampler.

actually filtered. Four collectors are usually attached to a single wire, and the end of wire is kept at certain depth by the depressing resistance of special depressor when being towed. Thus, the collection of the plankton from four layers is performed at the same time. Gauld and Beganal's net seems to be very simple and conventional. They made trial towing at speed of at least 7 knots.

The successive plankton sampler is an instrument of the same principle as the vertical sampler, but it is modified to tow at the surface from a moving ship. It is sturdily made of metal, consisting of a cylindrical tube and tapering portions at both ends, 10 cm in diameter of trunk, 100 cm in total length and 15 kg in weight. The head piece is made of solid metal to be particularly heavy, so as to sink when the instrument is towed at high-speed. As no special depressing vane is supplied, the resistance produced by the current in under-way as fast as 8 knots is only about 10 kg. At both sides of the head piece two holes, 2 cm in diameter respectively, are pierced. The water which entered from these holes is introduced into the condensing net, and the organisms left in this net are then stored in the posterior narrow cylindrical store-nets (1.5 cm in diameter and 18 cm in length) which are arranged circularly at the margin of the supporting disc. The magnitude of meshes of the cloth of the condensing and store-nets may be decided by the purpose of collection. The water straining from the large condensing net and the narrow store-nets flows out by the holes in the wall of the cylinder. The supporting disc of the store-nets is rotated very slowly by the motion produced by the posterior propeller when the instrument is towed, and so the organisms distributed along the course of the ship are successively stored in the different sotre-nets separately.

The distance of succession in sampling can be regulated to two kinds by changing the gear ratio (1/16000 and 1/80000). The experiments show that when the gear is connected as 1 : 16000 every store-net catches the organisms distributed along about 1 mile of the course at 8 knots ; that is one round of the supporting disc corresponds to 10 miles.

The present writer is greatly indebted to Prof. Dr. N. Inouye of the Faculty of Fisheries, Hokkaido University at Hakodate, for giving valuable suggestions in planning of the instruments. He is also very much obliged to Capt. S. Mishima of the Hokusei-maru, the training ship of the Hokkaido University, Capt. Imamura of the Soyo-maru, the surveying ship of the Tokai Regional Fisheries Research Laboratory, and Capt. M. Sato and Boatswain Kasai of Kaiyo-maru No. 4 of the Hydrographic Department, Maritime Safety Headquarters, for help in the trial sampling at sea. The present work has been rendered possible through the financial support of a grant-in-aid for Fundamental Scientific Research,

and another for Developmental Scientific Research, from the Ministry of Education, and partly through financial aid from the Hokkaido Fisheries Scientific Institution at Yoichi, for which the writer expresses his deep appreciation.

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（水産科学研究所業績　第121号）

SEASONAL DISTRIBUTION OF PELAGIC COPEPODS AT OSHORO BAY, WEST COAST OF HOKKAIDO.

by

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The routine work of plankton sampling at Oshoro Bay (Fig. 1), about 18 miles west of Otaru Harbour, on the west coast of Hokkaido, has been carried out usually every week, sometimes more unfrequently, throughout the year. The present studies are based on the samples collected from June, 1946, to December, 1950, of which the number of vials reaches to 162 in all. The sampling was made by horizontal haul of plankton net (27 cm in mouth diameter, made of bolting cloth, 128 meshes to an inch) for about 400 metres through a few metres depth in the bay, sometimes collections outside of the bay being also carried out.

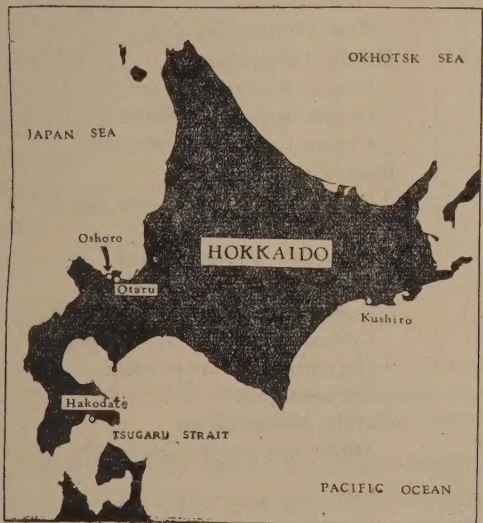


Fig 1. Sketch map of Hokkaido showing the situation of Oshoro Bay.

Fifty-six species of copepods were identified from the collections covering five years (Table 1), including thirty-one species of Gymnoplea and twenty-five species of Podoplea. Among them two Gymnoplea and one Podoplea are considered as new to science, about which a detailed account will be given in the near future. The characteristic feature of seasonal fluctuation of their occurrences is outlined as follows (attached Table, Fig. 2):

January: The community is monotonous, mainly composed of *Oithona similis* and *Paracalanus parvus*, which are distributed throughout the year. *Pseudocalanus minutus*, which has appeared from November of the preceding year, gradually increases in number toward the end of this month.

February: The community is the same as the preceding month, but the number of *Pseudocalanus minutus* greatly increases. The females of this species usually bear eggs.

Table 1 Systematic list of pelagic copepods collected at Oshoro Bay.

Suborder Gymnoplea
- Family Calanidae
  - *Calanus helgolandicus* (Claus)
  - *Calanus plumchrus* Marukawa
  - *Calanus tenuicornis* Dana
  - **Eucalanus bungii bungii* (Giesbrecht)
  - *Paracalanus parvus* (Claus)
  - *Paracalanus aculeatus* Giesbrecht
  - *Acrocalanus gracilis* Giesbrecht
  - *Clausocalanus pergens* Farran
  - *Clausocalanus furcatus* (Brady)
  - *Calocalanus pavo* (Dana)
  - *Calocalanus styliremis* Giesbrecht
  - *Calocalanus plumlosus* Claus
  - *Pseudocalanus minutus* (Kröyer)
  - *Euchaeta marina* (Prestandrea)
  - *Euchaeta ovata* Sato
- Family Centropagidae
  - *Centropages yamadai* Mori
  - *Centropages bradyi* Wheeler
  - *Centropgaes* sp.
  - *Pseudodiaptomus marinus* Sato
  - *Temora styrifera* (Dana)
  - *Temora discaudata* Giesbrecht
  - *Metridia lucens* Boeck.
  - *Lucicutia flavicornis* (Claus)
- Family Candacidae
  - *Candacia bipinnata* Giesbrecht
- Family Pontellidae
  - *Labidocera japonica* Mori
  - ***Acartia hamata* Mori
  - *Acartia clausi* Giesbrecht
  - *Acartia bifirosa* Claus
  - *Tortanus discaudatus* (Thompson and Scott)
  - *Tortanus forcipatus* (Giesbrecht)
  - *Tortanus* sp.

Suborder Podoplea
- Family Cyclopidae
  - *Oithona plumifera* Baird
  - *Oithona nana* Giesbrecht
  - *Oithona similis* Claus
  - *Oithona fallax* Farran
  - *Cyclopina longicornis* Boeck
  - *Setella gracilis* Dana
  - *Microsetella rosea* (Dana)
  - *Microsetella norvegica* (Boeck)
  - *Euterpe acutifrons* (Dana)
  - *Clytemnestra rostrata* (Brady)
  - *Harpacticus uniremis* (Kröyer)
  - *Laophonte* sp.
  - *Zaus spinatus* Goodsir
- Family Oncaeidae
  - *Oncaea venusta* Philippe
  - *Oncaea media* Giesbrecht
  - *Oncaea conifera* Giesbrecht
- Family Corycaeidae
  - *Sapphirina nigromaculata* Claus
  - *Copilia mirabilis* Dana
  - *Corycaeus speciosus* Dana
  - *Corycaeus agilis* Dana
  - *Corycaeus catus* F. Dahl
  - *Corycaeus anglicus* Lubbock
  - *Corycaeus concinnus* Dana
  - *Corycaeus* sp.
- Family Monstrillidae
  - *Monstrilla grandis* Giesbrecht

*This species is identical with *Eucalanus elongatus* var. *bungii* Giesbrecht 1892 (Pelagischen Copepoden, Fauna u. Flora d. Golfes v. Neapel, Vol. 19, p. 149) and to *Eucalanus giesbrechti* Mori 1937 (The pelagic copepoda from the neigbouring waters of Japan. p. 22, pl. 7, figs. 6-8). Johnson (1939, The study of species formation in certain *Eucalanus* copepods in the North Pacific. Proc. Sixth Pacific Congr., Vol. 3, p. 565) stated that *Eucalanus elongatus* var. *bungii* in the northern Pacific should be elevated to the rank of species. Wilson (1950, Copepods gathered by the United States Fisheries Steamer "Albatross" from 1887 to 1909, chiefly in the Pacific Ocean. Bull. U. S. National Museum, Vol. 14, Part 4, p. 208) accounted the Pacific specimens as *Eucalanus bungii* and Kokubo (1950, Rep. Surv. Fish. Resour. Aomori Bay, No. 1, p. 106) used the name of *Eucalanus bungii bungii* for the specimens of northern Japan.

**Preceding the late Wilson' s (1950, *loc. cit.* pp. 152–53, pl. 2, figs. 1–5) new species *Acartia hamata,* Mori (1937, *loc. cit.* pp. 104–105, pl. 51, figs. 1–5) gave a description of new species as *Acartia hamata,* to a species quite different from Wilson's species.

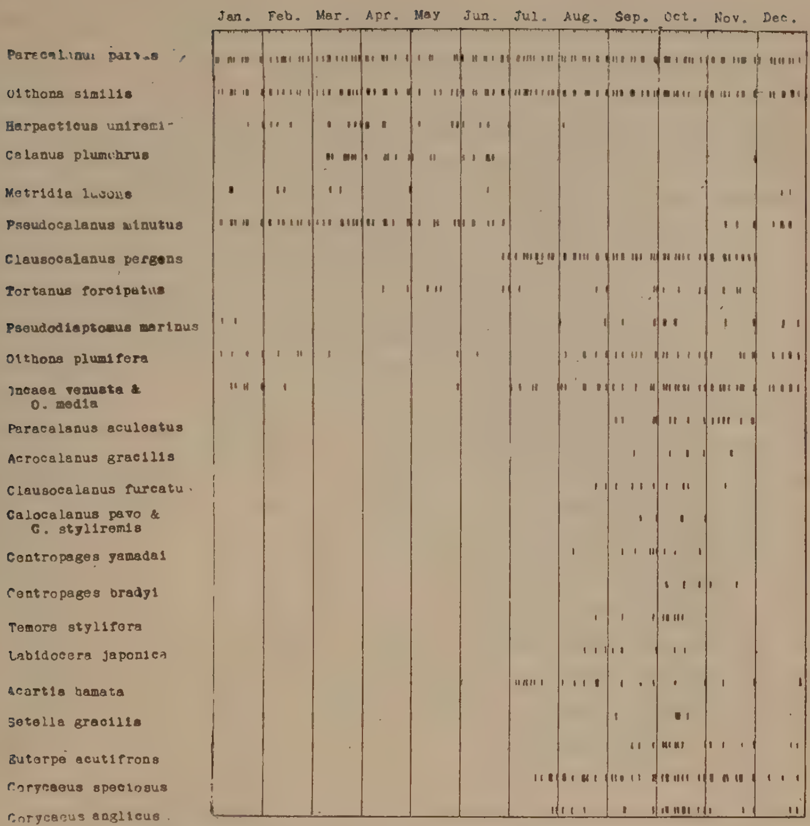


Fig. 2 General features of seasonal distribution of certain copepods based on successive five year observations.

March: Sudden appearance of an oceanic species, *Calanus plumchrus*, is observed in this month, and its appearance continues to about the end of June.

April: *Calanus plumchrus* becomes abundant, supplying the largest part of community.

May: The small-sized species, such as *Paracalanus parvus*, *Pseudocalanus minutus* and *Oithona similis*, are still observable.

June: *Paracalanus parvus*, is the main species, while *Pseudocalanus minutus* entirely disappears in the end of this month.

July: *Clausocalanus pergens* rapidly appears and increases in number to an overwhelming predominance. *Corycaeus speciosus* and *C. anglicus* appear in the middle of this month in some years. *Acartia hamata*, *Oncaea venusta* and *O. media*, also occur in this month.

August: At the beginning of this month *Pseudodiaptomus marinus*, *Oithona plumifera* and several species of *Corycaeus* appear; the former two species are distributed until the end of year or early the next year. *Labidocera japonica* occur at the middle of this month.

September: The community shows more diversity from the intermingling of tropical and subtropical species, such as *Paracalanus aculeatus, Temora stylifera, Setella gracilis, Euterpe acutifrons, Copilia mirabilis* and *Sapphirina nigromaculata.* Some of them have appeared from the preceding month in some years.

October: The above warm current forms still occur abundantly.

November: The warm current species disappear gradually toward the end of this month. A few males of *Pseudocalanus minutus* are collected in the bay at the month end anticipating their general appearance. The females of this species are found only in the off-shore collections in this month.

December: The warm current species almost disappear, though sometimes a few members remain behind. Both sexes of *Pseudocalanus minutus* and *Paracalanus parvus* increase in their number, but the total quantity of copepods in this month is very poor.

It is noticed that the copepod community in autumn includes various species of tropical and subtropical inhabitants, which migrate into the present area having been carried by the Tsushima Current. Iizuka (1950) has also pointed out that the subtropical *Ceratia* were found from October to November, not in the middle summer, at the present bay. Fish (1925) stated that the temperature is the dominant facter in governing the seasonal distribution of local pelagic animals of Woods Hole region. In the present bay, however, the occurrence of the oceanic warm current species is mainly influenced by the effect of the oceanic Tsushima Current, not being affected directly on temperature fluctuation. Thus the predominant appearance of tropical and subtropical species at the present bay lags about two months behind the period of highest temperature (late August) (Fig.3). The same tendency was observed by Kokubo (1946-50) at Aomori Bay into which an offshoot of the Tsushima Current flows.

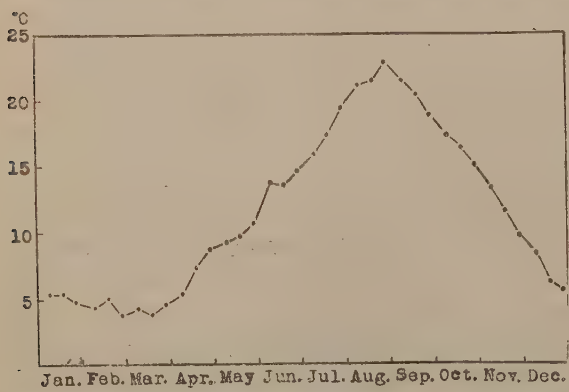


Fig. 3 Seasonal fluctuation of water temperature at Oshoro Bay averaging the data from 1946 to 1950 (Data were provided by Mr. Shinjiro Yamazaki).

The author (Motoda, Iizuka and Anraku, 1950) has assumed that the tropical and subtropical forms, which entered in the Japan Sea passing through the Chosen Strait in the middle summer, would appear at the northern part of the west coast of Japan in the autumn season, because the northward drift in the Tsushima Current from Chosen Strait to Hokkaido would require about two months. It is not yet confirmed whether the tropical and subtropical forms migrate into the Japan Sea from the south or not during the seasons other than the summer.

Yamada (1935) mentioned nine species, such as *Centropages calaninus, C. furcatus,*

Candacia catula, *C. aethiopica*, *C. curta*, *C. truncata*, *Labidocera acutifrons*, *Pontella securifer*, *P. atlantica*, as the characteristic copepods in the Tsushima Current, but all these species did not arrive at our coast.

At the west part of the Tsugaru Strait, Tsushima Current is divided into two flows. The northward current bathes along the west coast of Hokkaido, while the other passes eastward through the Tsugaru Strait into the Pacific Ocean. The tropical and subtropical oceanic copepods found at the present bay are 11 species, while those occurring at Aomori Bay (Kokubo, 1946-50) number 14 species (Table 2).

Table 2. The comparison of warm current copepods occurring at Aomori Bay (Kokubo, 1946-50) and Oshoro Bay.

Aomori Bay	Oshoro Bay
Calocalanus plumlosus	*Calocalanus plumlosus*
Calocalanus pavo	*Calocalanus pavo*
―――――	*Calocalanus styliremis*
―――――	*Sapphirina nigromaculata*
Copilia mirabilis	*Copilia mirabilis*
Paracalanus aculeatus	*Paracalanus aculeatus*
Acartia erythrea	―――――
Corycaeus curtus	―――――
Corycaeus speciosus	*Corycaeus speciosus*
Setella gracilis	*Setella gracilis*
Aetedius giesbrechti	―――――
Corycaeus rostratus	―――――
Euterpe acutifrons	*Euterpe acutifrons*
Labidocera acuta	―――――
Microsetella rosea	*Microsetella rosea*
Temora stylifera	*Temora stylifera*

The occurrences of *Clausocalanus pergens* and *Tortanus forcipatus* are confined to the months from July to November, while *Pseudocalanus minutus* is collected during the cold season from November to June. The latter species, however, occurs from January to June at Aomori Bay (Kokubo, 1946-50) and from March to September in the Gulf of Maine and Bay of Fundy (Fish, 1936).

Both the number of species and the quantity are more abundant outside of the bay than in the bay. This is also the case of *Ceratia* (Iizuka, 1950).

Besides the above, semibenthic copepods, *Harpacticus uniremis*, *Laophonte* sp., *Zaus spinatus* and the larval form of parasitic copepod, *Monstrilla grandis*, are collected, but the

latter three species occur only occasionally.

The author wishes to express his hearty acknowledgement to Prof. Dr. Sigeru Motoda, Faculty of Fisheries, Hokkaido University, Hakodate, for painstaking guidance through his work. He also wishes to express deep thanks to Mrs. Tamezo Yamazaki and Kazuro Shida, members of the Oshoro Marine Biological Station for laborious work of collection at sea.

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（水産科学研究所業績　第122号）

STUDIES ON SHARK MUSCLE. PART 1. HISTOCHEMICAL STUDIES OF UREA IN SHARK MUSCLE.

Keiichi OHOISHI

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Since 1858, the presence of a large amount of urea in shark muscle has been known, but histochemical studies have not yet been reported. At the begining of this shark muscle study, therefore, histochemical investigations have been carried out. In this paper, the position of urea in the microscopical shark muscular tissue and the crystal form of urea are described.

Experimental

Two sharks were used as the experimental materials. The one was a female of "Hoshizame" fish *Mustelus manazo* BLEEKER taken off Maizuru on April 23rd, 1951, 85cm. in total length and 2,850 g. in body weight; several young fish were in its embryo sac. The other was a male of "Dochizame" fish *Triakis scylliun* MUELLER *et* HENLE which was taken at the same place, on June 13th, 1951, 90 cm. in total length and 1,850 g. in body weight. The dorsal muscles of these fish were cut off in 1 cm. cube blocks, and fixed by the STUEBEL method or OLIVER method [1]; in this process the urea is crystalized as dixanthylurea. These blocks, after imbedding with carbowax, were cut 5μ in thickness, stained with heamatoxylin and eosin, then examined microscopically. Results are shown in the following plates.

In any specimen, cross striation, nucleus and sarcolemma of muscular fiber are shown. From plates 1 to 8, they are cited to the rank of crystal size. Crystals in the center of specimens are smaller in size and less in number than those on the outside, though the cause of this difference is not completely explainable. In plates 9 and 10, is shown a group of crystals. Such a gathering is found on the outside of specimens. This transfer of urea to the outside in crystallizing as dixanthylurea from the inside tissue, is also presumed as in the previous paper [2]. The presence or absence of urea in the musculer fiber has not been obvious till now. But, the sarcolemma is stressed by the formation of crystal as shown in plate 11, and broken down by the stressing of crystal in plate 12. Observing these results, it may be considered that urea is present in the shark muscular fiber after crystallizing as dixanthylurea, though its presence in natural state is unknown, as heretofore.

Summary

Urea is found in the shark muscular fiber as far as examined as dixanthylurea; the size of such crystals was smaller and their number less on the inside as compared with outside

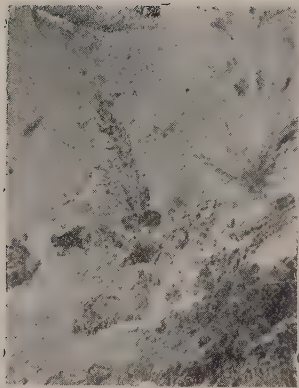


Plate 1. *Mustelus manazo.*
Fixed on the catching day
by the Oliver method. 62×10

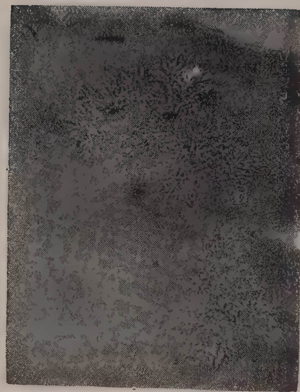


Plate 2. ditto



Plate 3. ditto

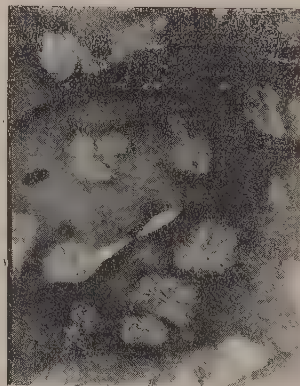


Plate 4. ditto 40×15

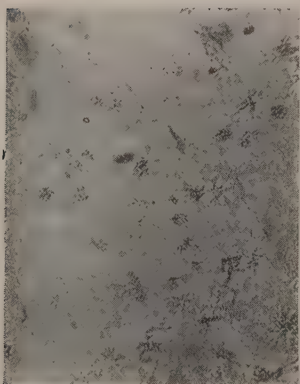


Plate 5. ditto 62×10

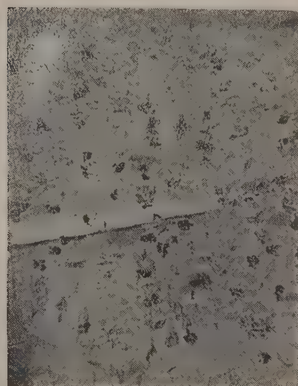


Plate 6. ditto

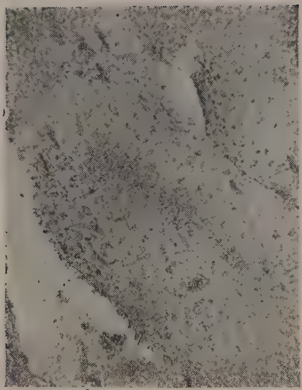


Plate 7. ditto



Plate 8. *Triakis scyllium.* Fixed after 17days of catching by the Stuebel method. 62×10

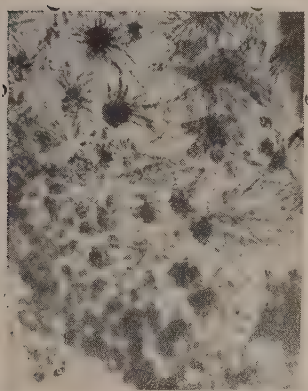


Plate 9. *Triakis scyllium.* Fixed after 8 days of catching by the Stuebel method. 62×10

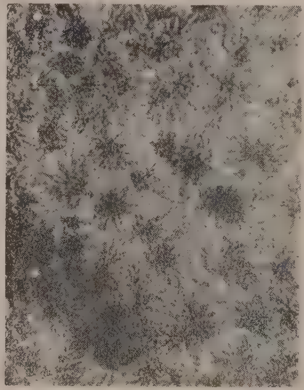


Plate 10. ditto

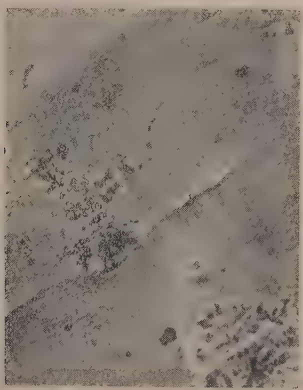


Plate 11. *Mustelus manazo.* Fixed on the catching day by the Oliver method. 62×10

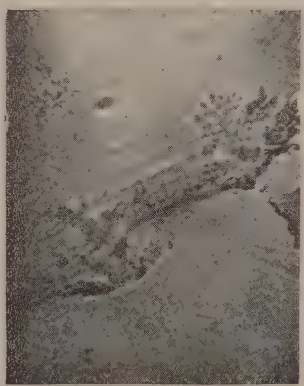


Plate 12. ditto

of these specimens.

Literature cited

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(2). K. Ohoishi (1952): *Kagaku,* Vol. 22, 424.

（水産科学研究所業績　第123号）

ON THE MODE OF ENZYMIC HYDROLYSIS OF PROTEIN. PRELIMINARY. ON THE TRYPTIC DIGESTION OF WHOLE CASEIN.

Shuichiro KUBO

(Faculty of Fisheries, Hokkaido University.)

Protein, of which the molecular structure is extremely complicated, has a unique significance in biology. On the other hand, each proteinase has definite specificity of substrate, protein, and hydrolyzes such peptide bonds as are present in the substrate in a certain structural composition. Therefore the specific structure of protein is an expression of the specificity of the enzyme which hydrolyzes the substrate. It follows that, in order to define the structure of protein, it may be quite important to study the mode of enzymic hydrolysis of protein and the specificity of proteinase.

On these two points extensive studies by Bergmann and Fruton[(1),(2),(3)] have yielded information about synthetic peptides, since Michaelis' concept of the enzyme-substrate compound has been proposed, it has been assumed that this combination would result in the activation, and subsequent hydrolysis, of an adjacent peptide bond of the substrate. The net charge of protein appears to be changed by this combination.

As an investigation along this line, electophoretic study of protein hydrolysis in earlier stages of digestion is reported in this paper. Some investigation of peptic digestion of egg albumin along this line has been previously made by Tiselius and Eriksson[(4)] at optimum temperature. But the digestion at optimum temperature seemed to have the high degree of digestion and to be not suitable for electrophoretical analysis of digestion product. Accordingly digestion at 4°C was employed in tryptic digestion of whole casein in this work.

EXPERIMENTAL

1. Materials

Whole casein was reprepared according to the method of Hammarsten. It contains three components in electrophoretic patterns.

The enzyme used was trypsin powder (made by Wako Company); it was not crystalline.

Table 1. Composition of materials.

Fraction	Total-N %	Total-NH_2-N %	Free-NH_2-N %
Whole casein	13.917	11.219	0.880
Trypsin	15.127	—	3.772

2. Measurements

Electrophoretic analysis was proceeded by HT-B type Tiselius Apparatus (Hitachi

Seisakusho Company) and the scale of cell was 2 × 15 × 50 mm. On the observation of patterns, the ascending boundary was adopted, which seemed to give stable and sharp boundary in casein.[5] Micro Kjeldahl method was used for the determination of total nitrogen, and the degree of hydrolysis was determined by Van Slyke's.

3. Samples

In electrophoretic analysis, specific refractive increment is a very important factor to decide a component according to optical method, and moreover it is affected by the thickness of cell. A small cell, 2 × 15 ×50 mm. should be used; it might be impossible even at 5.461 Å of wave length to determine any component, which has a smaller specific refractive increment than that of 3×10^{-5}.[6] As the specific refractive increment of protein is 0.0018~0.0019 / g. in ordinary, no boundary appears when the amount of protein in 15 mm. cell is less than about 0.015 g. / 100ml.

And so in this experiment, the amounts of trypsin were adjusted so as not appear in boundary.

One gm. of casein was dissolved in 90 ml. of each pH phosphate-borax buffer (μ=0.10), and casein solution was dialyzed for the same buffer for two days at 4°C. (sample : buffer=1 : 50 in volume).

0.09 g. of trypsin was dissolved in 100 ml. each pH phosphate-borax buffer (μ= 0.10) and the supernatant solution to be acquired from centrifuged trypsin solution was dialyzed for the same buffer for two days at 4°C. (sample : buffer= 1 : 50 in volume).

Composition of sample :

Table 2. Casein

Fraction	pH 7	pH 8	pH 9
Casein g./100ml.	0.968	0.990	0.996
Total-N mg./100ml.	134.78	137.81	138.67
Free-NH_2-N mg./100ml.	8.52	8.71	8.76

Table 3. Trypsin

Fraction	Trypsin g./100ml.	Total-N mg./100ml.	Free-NH_2-N mg./100ml.
Trypsin pH7,8,9	0.090	13.63	3.39

In digestion, one part of trypsin solution wes added to nine parts of casein solution, trypsin solution became 0.009 g trypsin per casein solution 100ml. and did not appear in boundary.

4. Digestion at 37°C

In order to know the optimum pH of this trypsin and the state of digestion, preliminary investigation was made at 37°C. Three ml. of trypsin solution was added to 27 ml. of casein solution and was digested at 37°C.

The obttained results were as shown in Table 4.

It is evident that the optimum pH of this trypsin to casein was pH 9 and the next pH 8, as table 4 B shows.

5. Digestion at 4°C.

As the digestion at 37°C seemed to indicate a high degree of digestion and to be not suitable for electrophoretical analysis in studying the digestion of casein with trypsin, digestion at 4°C was carried through in the same manner at 37°C.

Table 4. Digestion of casein with trypsin at 37°C.

A. Free NH_2-N mg per 100 ml. sample.

Fraction / Time (hour)	pH 7	pH 8	pH 9
0	7.67	7.84	7.89
½	10.29	14.84	16.24
1	11.75	18.06	20.15
1½	13.93	22.31	26.23
2	16.29	26.07	33.32
2½	18.65	29.51	39.83
3	21.75	33.34	43.62
3½	25.04	37.79	45.38
4	28.46	40.73	45.39
4½	31.74	42.03	45.39
5	33.92	42.05	—
5½	35.25	42.06	—
6	35.53	—	—

B. Per cent of free NH_2-N for total NH_2-N.

Fraction / Time (hour)	pH 7	pH 8	pH 9
0	7.84	7.83	7.84
½	10.52	14.84	16.14
1	12.01	18.06	20.02
1½	14.24	22.31	26.06
2	16.65	26.07	33.11
2½	19.07	29.51	39.58
3	22.24	33.34	43.35
3½	25.60	37.79	45.10
4	29.10	40.73	45.11
4½	32.45	42.03	45.11
5	34.68	42.05	—
5½	36.04	—	—
6	36.33	—	—

The results shown in this table were the average of three digestions; free NH_2-N of trypsin was subtracted.

As table 5A and B shows, the amounts of free NH_2-N had decreased in addition of trypsin solution to casein, before it was increased by digestion. The decreasing ratio of NH_2-N was the greatest in optimum pH 9, the next at pH 8.

6. Electrophoretic analysis of tryptic digestion of casein at 4°C.

The partially digested casein solution was investigated in the HT-B type Tiselius Apparatus (Hitachi Seisakusho Company) at 4°C.

At first, dialyzed casein and trypsin solution, which were previously described under "Samples", were mixed at the ratio of 9 : 1, and digested at 4°C for the expected time. Digested casein solution was immediatly electrophoryzed in each pH phosphate-borax buffer (μ=0.10), so that some low molecular material which was produced in digestion would not dialyze out of the bag. But the accurate measurement of mobilities could not be expected. The patterns obtained were as shown in Fig. 1.

Table 5. Digestion at 4°C of casein with trypsin.

A. Free NH_2-N mg per 100ml. sample.

Time (hour) \ Fraction	pH 7	pH 8	pH 9
0	7.67	7.84	7.89
Addition with trypsin	5.44	4.75	2.48
½	4.75	4.19	5.03
1	2.48	2.48	5.88
2	3.30	4.23	6.78
3	4.12	5.75	7.69
4	4.97	6.28	8.60
5	5.54	6.62	9.50
6	5.92	7.01	10.38
9	7.00	8.12	11.26
12	8.15	9.27	12.10
15	9.26	10.40	12.66
18	10.41	11.54	13.28

The results tabulated here were the average of three digestions; free NH_2-N of trypsin was subtracted.

B. Per cent of free NH_2-N for total NH_2-N.

Time (hour) \ Fraction	pH 7	pH 8	pH 9
0	7.84	7.83	7.84
Addition with trypsin	6.01	4.75	2.46
½	4.85	4.19	4.99
1	2.53	2.43	5.84
2	3.37	4.23	6.73
3	4.21	5.75	7.64
4	5.08	6.23	8.54
5	5.66	6.62	9.44
6	6.05	7.01	10.31
9	7.15	8.12	11.19
12	8.33	9.27	12.02
15	9.46	10.40	12.58
18	10.64	11.54	13.14

DISCCUSION

Fig. 2. and Fig. 3 are illustrated by Table 4B and Table 5B.

As shown by Fig. 2, optimum pH of this digestion at optimum temperature was pH 9, the second pH 8, and the last pH 7. The digestion at 4°C had the same tendency, but in earlier stage of digestion. it was different from the digestion at 37°C. That is, owing to the addition of trypsin. the free NH_2-N of casein solution decreased suddenly, and the speed of that decreasing tendency in respect to time was in proportion pH. Moreover, the minimum point of decreasing free NH_2-N was about the same in each pH.

It may be supposed that the decrease is due to a combination of trypsin with the substrate, casein, as in the Michaelis′ concept of the enzyme-substrate compound. That is to say, trypsin combines free NH_2-groups of side chain in casein and this combination would result in the activation, and subsequent hydrolysis, of an adjacent peptide bond of the casein.

The digestion at 4° C is so slow in comparison with that at 37° C, that this decreasing

Fig. 1. Electrophoretic patterns of partially digested casein at 4°C.



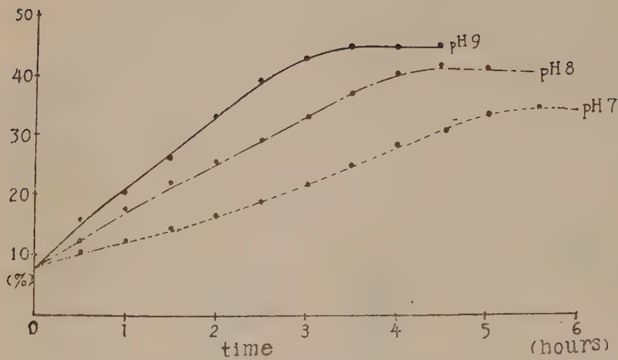


Fig. 2. Digestion at 37°C of casein with trypsin (per cent of free NH_2-N for total NH_2-N).

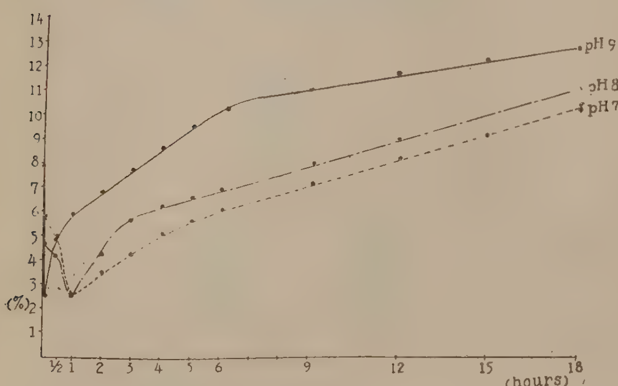


Fig. 3. Digestion at 4°C of casein with trypsin (per cent of free NH_2-N for total NH_2-N).

may be observed.

The calculated results of electrophoretic analysis were as shown in Table 6.

" Digestion 0" in Table 6 and Fig. 1 show the time of addition of trypsin, but electrophoretic time was 2280 sec. (38 minutes), then digestion may be progressing in this period. According to Fig. 3, digestion at pH 8 and pH 7 decreases in this period, but that of pH 9 increases. When trypsin had been added to casein solution "Digestion 0", a new component β_2 appeared. It is not exactly certain that β_2 may be enzyme substrate combination, but a slight preliminary change before casein was broken down to small particles was observed and the ratio of produced β_2 was the greatest in the pH 9 sample.

According to the increase in the digestion, high mobility components and low mobility-components which are both inhomogeneous, were produced. In addition to that, the amounts of low mobility products were greater than those of high mobility products and the ratio of these products was the greatest in pH 9 sample.

In fraction α, component α_2 did not change the mobility. It has not been broken down to small particles, which are considered unchanged α - casein. In the case of digestion of casein ,at 4°C , a few molecules should be attached and solwly broken down to the end-products.

According to the mechanism of enzymic proteolysis as described By Fruton, [7] it would be expected that at first trypsin combines with the side chain of substrate and whereas trypsin which can approach the peptide bond of substrate split the peptide bond.

SUMMARY

In order to learn the mode of enzymic proteolysis, the study of tryptic digestion on

Table 6. The components and mobilities of digested casein.

Digestion time (hours)	Fraction	pH 7 No.	pH 7 α	pH 7 β	pH 7 γ	pH 8 No	pH 8 α	pH 8 β	pH 8 γ	pH 9 No.	pH 9 α	pH 9 β	pH 9 γ
Blank	ratio (%)	70	64.3	13.6	22.1	80	62.5	13.8	22.7	90	82.7	13.7	23.6
	mobility		8.4	5.1	−0.5		9.3	5.9	−0.6		9.8	7.1	1.0
0	ratio (%)	71	58.3	β_1 16.6 β_2 5.0	20.1	81	52.9	β_1 10.5 β_2 11.9	24.7	91	α_1 3.3 α_2 49.9	β_1 13.4 β_2 13.1	20.3
	mobility		8.5	5.1 2.9	−0.7		9.3	6.4 3.6	0.2		10.7 9.9	6.6 3.5	0.3
6	ratio (%)	72	α_1 10.4 α_2 36.5	β_1 13.9 β_2 11.4	27.8	82	α_1 18.3 α_2 35.3	β_1 10.6 β_2 8.2	27.6	92	α_1 7.5 α_2 33.7	β_1 12.5 β_2 18.7	27.6
	mobility		9.9 8.5	5.2 2.7	−0.8		10.0 9.8	6.2 3.5	−0.2		10.8 10.0	6.3 3.3	−0.5
12	ratio (%)	73	α_1 11.2 α_2 35.0	β_1 13.2 β_2 9.8	30.8	83	α_1 11.5 α_2 34.7	β_1 14.7 β_2 9.0	30.1	93	α_1 22.4 α_2 30.0	β_2 17.0	30.6
	mobility		10.2 8.6	5.6 2.7	−0.3		10.1 9.4	6.3 3.4	−0.7		11.4 10.0	3.4	−0.6
18	ratio (%)	74	α_1 10.8 α_2 33.3	β_1 18.0 β_2 7.3	30.6	84	α_1 17.6 α_2 32.3	β_2 8.4	41.7	94	α_1 (α_1' 7.0 α_1'' 17.6) α_2 28.3	β_2 12.1	46.9
	mobility		10.6 8.7	5.2 2.8	−0.4		11.4 9.5	3.4	0.3		12.6 11.0 10.0	3.3	−0.2

mobility : $10^{-5} cm^2/volt.\ sec.$

whole casein at 4°C was carried out by Electrophoresis and by the determination of free NH_2-N. The results obtained were as follows.

1) In early stage of digestion, when trypsin solution was added to casein solution, the free NH_2-N of casein decreased suddenly (Table 5B and Fig.3).

2) The decrease seems to be due to the fact that trypsin combines free NH_2- groups of side chain in casein.

3) With electophoretic analysis, a slight preliminary change before casein was broken down to small particles was observed and the ratio of the new component was the greatest in optimum pH (Table 6 and Fig. 1).

4) In accordance with the increase in digestion, high mobility-components and low mobility-components both inhomogeneous, were produced. (Fig. 1 and Fig. 6).

The author wishes to express his hearty thanks to his respected teacher, Prof. Katsuzi Yoshimura for his helpfull guidance through out in this work.

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（水産科学研究所業績　第124号）

「スルメイカ」の生化學的研究

第1報 「スルメイカ」筋肉及びエキスの窒素形態並にアミノ酸に就て

吉村克二・久保周一郎 （水産生物化学教室）

BIOCHEMICAL STUDIES ON "SURUME-IKA" (*Ommastrephes sloani pacificus*).

I. NITROGEN FRACTION AND CONTAINED AMINO ACIDS IN MUSCLE AND IN EXTRACTED MATTER WITH WATER.

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"Surume-ika" (*Ommastrephes sloani pacificus*) is belong to Mollusca, Sephalopoda. It has the giant nerve fiber and specific form; moreover its muscle is glassy and rather soft.

In this paper, biochemical observations of "Surume-ika" made at different seasons were reported. Data were obtained on the nitrogen-fraction and contained amino acids in muscle and in extracted matter with water. The results are summarized as followes.

1) On the weight-ratic of trunk and head of "surume-ika" of summer were greater than those of autumn, but the organs were the opposite.

2) On the difference of nitrogen-fraction, the amounts of insoluble-N, total-N in basic form and basic amino acids of "surume-ika" of autumn were greater than those of summer.

3) As to the kinds of amino acids, fifteen kinds were recognized in trunk, but not leucine in head. Differences by season were not recognized.

4) As the nitrogen-fraction in extracted matter with water, many nitrogen-fractions without amino acids were contained, which seemed to be amine, purine bases and betain. Nine kinds of amino acid were recognized.

「スルメイカ」は軟体動物頭足類に属し活動的動物にして、殊に其の神経繊維は他動物に比し太く形態的にも特異な形狀をなして居る。筋肉も柔軟性で且つ平滑であり生化学的にも特性あるものと予想される。

イカの日本に於ける漁獲高は9000万貫に及びイワシに次ぎ第2位で全国に亘り殆ど周年漁獲せられるが、其の大部分は北海道の沿岸特に函館を中心とする道南に於て漁獲せられる。現在日本に於ける最も不足せる動物性蛋白質の給源として重要なものである。

「シリヤケイカ」に附いては先に波多腰氏[(1),(2),(3)]の詳細な研究があるが、道南地方に於て多獲せられる「スルメイカ」に附いては確たる研究結果がないので「スルメイカ」に附き先づ筋肉及びエキス中に含有せられる窒素形態並にアミノ酸の種類を夏イカ（8月漁獲せるもの）、秋イカ（10月漁獲せるもの）に就き定量した。

I. 実 験 の 部

1. 試　　料

夏イカ：8月3日函館近海に於て漁獲せられたもので体各部分の重量比並に其の蛋白質含有量は次の第1表の如くである。

Table 1. Weight-ratio and protein contents of "Surume-Ika" of Summer

A. Weight-ratio

Fraction	Total Weight	Trunk	Head	Organs
Weight(g.)	225	125	50	50
Pre cent	100	55.54	22.23	22.23

B. Protein contents

Fraction	Moisture	Crude protein	The rest
Trunk	77.13	21.66	1.21
Head	78.76	16.90	4.34

Table 2. Weight-ratio and protein contents of "Surume-Ika" of Autumn.

A. Weight-ratio

Fraction	Total weight	Trunk	Head	Organs
Weight(g.)	290	150	80	60
Pre cent	100	51.73	27.58	20.69

B. Protein contents

Frection	Moisture	Crude protein	The rest
Trunk	76.09	22.20	1.71
Head	77.13	18.77	4.10

秋イカ：10月17日函館近海に於て漁獲せられたもので体各部分の重量比並に其の蛋白質含有量は第2表の如くである。

試料として使用した夏イカと秋イカを比較すると夏イカは秋イカに比して胴部並に頭足部が多く内臓量が少い。蛋白質含有量は胴部頭足部共に秋イカが大であつて水分の含有量は少ない。

2. 筋肉の窒素形態

夏イカ及び秋イカの胴部、頭足部に就きバンスライク氏法に依り其の窒素形態を見たが其の結果は第3表、第4表の如くである。

以上夏イカ及び秋イカ胴部、頭足部の窒素形態を見ると先ず全窒素量は秋イカの方が僅かに多い。之は夏イカの方が水分の含量大なるため当然の結果と考えられる。塩酸不溶性窒素は夏イカより秋イカの方が多量となり特に頭足部に於て其の含有量が多い。アマイド態窒素は秋イカが少いが之は筋肉蛋白質の縮合度が進む事に依り当然考えられることである。塩基性窒素は秋イカの方が多く且つ塩基性アミノ酸の各部全般に亘り増加して居る。

胴部に比し頭足部は夏イカ、秋イカ共に各成分の含有量は少い。

筋肉を構成して居るアミノ酸含量の分布は時期に依り変化のある事が認められる。

3. 筋肉のアミノ酸の種類

夏イカ及び秋イカに就き胴部、頭足部を夫々20%の塩酸で分解し其の分解液に附きフエノール、ルチジンを溶媒としてペーパークロマトグラフ法に依りアミノ酸の種類を見た。其の結果は次の第1図及び第2図の如くである。

Table 3. Nitrogen fraction of "Surume-Ika" of Summer

Fraction	Trunk		Head	
	In 100g (gr)	Per cent for total-N	In 100g (gr)	Per cent for total-N
Total-N	3.467	100	2.704	100
Insoluble-N for 20% HCl	0.008	0.24	0.023	0.89
Amid-N	0.414	11.74	0.306	11.33
Humin-N	0.090	2.60	0.054	1.99
Total N in form of bases	1.276	36.80	0.803	29.69
Arginine-N	0.519	14.97	0.294	10.87
Histidine-N	0.413	11.91	0.262	9.69
Lysine-N	0.311	8.97	0.247	9.13
Cystine-N	0.034	0.98	0.031	1.14
Total-N in form of mono amino fraction	1.657	47.79	1.505	55.69
Amino N in form of mono amino fraction	1.294	37.32	1.243	45.97
Total *	3.445	99.37	2.691	99.57

* Insoluble-N for 20% HCl+Amid N+Humin-N+Total-N in form of bases + Total-N in form of mono amino fraction.

Table 4. Nitrogen fraction of "Surume-Ika" of Autumn

Fraction	Trunk		Head	
	In 100gr. (gr.)	Per cent for total-N	In 100gr (gr.)	Per cent for total-N
Total-N	3.552	100	3.004	100
Insoluble-N for 20% HCl	0.069	1.97	0.035	1.17
Amid-N	0.341	9.61	0.307	10.22
Humin-N	0.119	3.35	0.068	2.26
Total N in form of bases	1.350	38.00	1.002	33.36
Arginine-N	0.595	16.75	0.351	11.68
Histidine-N	0.364	10.25	0.311	20.32
Lysine-N	0.342	9.63	0.291	9.69
Cystine-N	0.039	1.10	0.049	1.63
Total-N in form of mono amino fraction	1.664	46.84	1.582	52.66
Amino-N in form of mono amino fraction	1.283	36.09	1.262	42.01
Total *	3.543	99.77	2.994	99.67

* Insoluble-N for 20%HCl + Amide-N + Humin-N + Total-N in form of bases + Total-N in form of mono amino fraction.

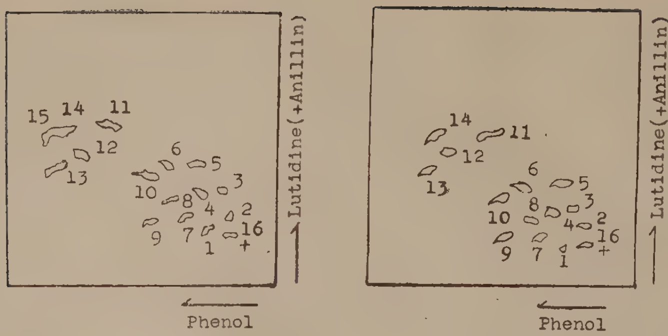


Fig. 1. Kind of amino acid in "Surume-Ika" of Summer.
A. Trunk (Hydolrysis with 20% HCl).
B. Head (Hydrolysis with 20%HCl).

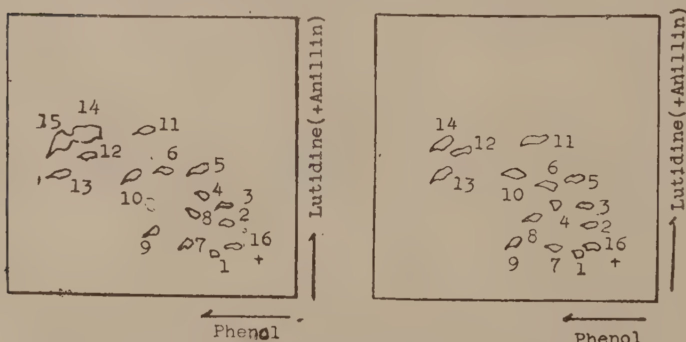


Fig. 2. Kind of amino acid in "Surume-Ika" in Autumn.
A. Trunk (Hydrolysis with 20%HCl).
B. Head (Hydrolysis with 20%HCl).

1. Asparagic acid	9. Arginine
2. Glutamic acid	10. Alanine
3. Serine	11. Tyrosine
4. Glycine	12. Valine
5. Taurine	13. Proline
6. Threonine	14. Leucine
7. Lysine	15. Isoleucine
8. Histidine	16. Cystine

即ちトリプトフアンは塩酸で分解せられる為め認める事が出来なかつたが、アスパラギン酸、グルタミン酸、セリン、グリシン、スレオニン、リジン、ヒスチジン、アルギニン、アラニン、チロシンバリン、プロリン、ロイシン、イソロイシン、シスチンの15種のアミノ酸の外にタウリンを認める事が出来た。アミノ酸の種類に於ては時期に依る差違はないが胴部、頭足部を比較する時は夏イカ、秋イカ共に頭足部にはイソロイシンを缺いて居つた。

4. 「エキス」中の窒素形態

生イカの内臓を除去したものを温水で繰返し抽出し抽出物を適当に濃縮し、三塩化醋酸を加えて可溶性蛋白質を除去したものに就きバンスライク氏法に依り窒素形態を測定した。其の結果は第5表及

Table 5. Nitrogen fraction in extracted matter of "Surume-Ika" of Summer.

Fraction	In extracted matter of 100gr of "Surume-Ika" (gr)	Per cet for total-N in 100gr of "Surume-Ika"	Per cent of total-N
Total-N	0.647	19.91	100
Amide-N	0.030	0.92	4.56
Humin-N	0.064	1.97	9.94
Total-N in form of bases	0.232	7.14	35.85
Arginine-N	0.016	0.34	1.70
Histidine-N	—	—	—
Lysine-N	—	—	—
Cystine-N	0.002	0.06	0.37
Total-N in form of mono amino fraction	0.306	9.42	47.36
Amino-N in form of mono amino fraction	0.058	1.78	8.95

Table 6. Nitrogen fraction in extracted matter of "Surume-Ika" of Autumn.

Fraction	In extracted matter of 100gr of "Surume-Ika" (gr)	Per cent for total-N in 100gr of "Surume-Ika"	Per cent for total-N
Total-N	0.642	19.10	100
Amide-N	0.018	0.54	2.85
Humin-N	0.101	3.00	15.71
Total-N in form of bases	0.257	7.65	40.04
Arginine-N	0.016	0.48	2.44
Histidine-N	—	—	—
Lysine-N	—	—	—
Cystine-N	0.016	0.48	2.44
Total-N in form of mono amino fraction	0.264	7.85	41.19
Amino-N in form of mono amino fraction	0.061	1.81	9.44

び第 6 表の如くである。

以上の結果を見るに先づエキス量は夏イカが秋イカに比して幾分多い。而してエキス中に出て来る窒素はアミノ酸以外の形態の窒素が多い。之はアミン、ピュ－リン塩基、ベテイン其の他の形態のものと思われる。

5. 「エキス」中のアミノ酸の種類

ペーパークロマトグラフ法に依りアミノ酸の種類をみた結果は第 3 図及び第 4 図の如くであつて之より見るに 9 種のアミノ酸即ちアスパラギン酸、グルタミン酸、グリシン、アルギニン、リジン、アラニン、バリン、プロリン、ロイシンを認める事が出来た。此の外にタウリンの存在も確認する事が出来た。筋肉蛋白質に認められたスレオニン、シスチン、セリン、ヒスチヂン、チロシン、イソロイシンは之を認める事が出来なかつた。奥田氏はヤリイカのエキス中にヒスチヂン、チロシン[4]の存在を認めて居るが之は認め得なかつた。

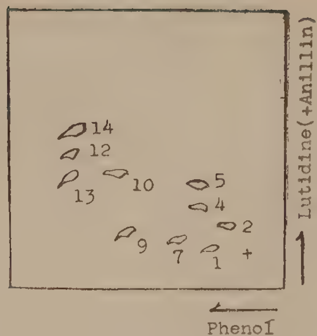


Fig. 3. Kind of amino acid in extracted matter of "Surume-Ika" of Summer.

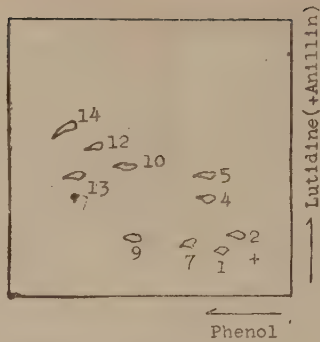


Fig. 4. Kind of amino acid in extracted matter of "Surume-Ika" of Autumn.

1. Aspartic acid
2. Glutamic acid
4. Glycine
5. Taurine
7. Lysine
9. Arginine
10. Alanine
12. Valine
13. Proline
14. Leucine

Ⅱ. 摘　　要

(1) 「スルメイカ」に附いて胴部、頭足部及びエキスに含有される窒素形態並にアミノ酸の種類を夏イカ、秋イカに就いて定量した。

(2) 「スルメイカ」の体各部の重量比を見るに一般に夏イカは秋イカに比し胴部及び頭足部が多く内臓は少い。且蛋白質含量は秋イカの方が大であつた。

(3) 胴部、頭足部の窒素形態の季節的差異は秋イカの方が塩酸不溶性窒素、塩基性窒素及び塩基性アミノ酸が夏イカに比して大である。

(4) 含有アミノ酸の種類に於ては胴部の塩酸加水分解物中には15種のアミノ酸及びタウリンが認められた，頭足部に於てはイソロイシンを缺いて居つた，且季節的差異は認められなかつた。

(5) エキス分の窒素形態はアミノ酸以外の形態の窒素が多く之れはアミン、ピユーリン塩基、ベテイン等に依るものと思われる。

(6) エキス中には9種のアミノ酸及びタウリンが認められたがヒスチヂン、チロシンは認められなかつた。且季節的差異も認められなかつた。

文　　献

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（水産科学研究所業績　第125号）

「スルメイカ」の生化學的研究

第2報　塩基性アミノ酸の含量について

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BIOCHEMICAL STUDIES ON "SURUME-IKA" (*Ommastrephes sloani pacificus*).

THE CONTENT OF BASIC AMINO ACID.

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1. The author has attempted to ascertain the nutritional value and position of "Surume-ika" (a kind of squid, *Ommastrephes sloani pacificus*) meat as food.

2. Analysis of the "Surume-ika" meat have been made to determine the content of basic amino acid by isolation of amino acid derivatives described by Vickery and Block, and Block. Histidine and arginine were isolated by precipitation with flavianic acid and lysine was separated by precipitation with picric acid.

3. According to the data, "Surume-ika" meat contains 1.2～1.4% histidine, 4.8～7.5% arginine and 4.6～7.0% lysine in each state. In the distribution of basic amino acid nitrogen, arginine nitrogen shows the highest value.

4. The amount of the basic amino acid is the same as that found in other muscle and fish meat. Therefore, it is apparently indicated that the nutritional value of "Surume-ika" is not inferior to that of other meat.

5. But, to learn more exactly the nutritional value the content of other essential amino acids must be determined and the results obtained may depend on the breeding test of animals.

緒　　言

前報告[1]に於てはスルメイカ肉の窒素分布及びアミノ酸の存在を研究し、定性的には15種のアミノ酸及びタウリンが存在すること、エキス中には9種のアミノ酸及びタウリンの存在することを明かにした。

イカ肉の栄養価は消化率と、存在する重要アミノ酸の含有量によるので、特に塩基性アミノ酸なるアルギニン、リジン、ヒスチヂンが問題となる。奥田氏がヤリイカに付て調べたのを見ると、アルギニン含有量は他の魚肉、獣肉に比し甚しくその含量が大で、ヒスチヂンはそれほど差違なく、リジンは牛肉、鶏肉、鯨肉に比し多少は少いが他の魚類に比しては必ずしも少ないとは考えられない。軟体類の他の動物に比しては含有量が多い。スルメイカについても之等の含有量を明かにしその栄養價値を知る必要あり之等の食品を定量すると共に其の時期的に如何なる消長があるかを明かにせんとして

次の実験を行つた。

実　験　の　部

試料は函館近郊で夏及び秋に捕獲されたスルメイカ（*Ommastrephes sloani pacificus*）を風乾せるスルメ製品を細切して用いた。

試料の一定量（2.5瓦の蛋白質に相当する量）をとり、約10倍量の8規定硫酸で24時間逆流冷却器を附して加水分解する。分解終了後不溶物を濾過し熱水洗滌し濾液及び洗液を合し、それに熱した飽和水酸化バリウム溶液を加えてコンゴーロートで pH 3.5に中和し、一定量(50 cc.)に減圧濃縮する。この中和濃縮したアミノ酸加水分解液について塩基性アミノ酸の定量を行つた。

塩基性アミノ酸の定量は多くの研究者により行われているが、こゝでは Vickery の改良法[2] 及び Block[3] 法を基礎として互に組合せて行つた。原理はヒスチヂンは pH 7.4 で銀塩として沈澱しアルギニンは pH 10 以上で銀塩として除去し、銀で沈澱しない部分がリジンになる。各々この分離したアミノ酸をフラビアナート及びピクラートとして分離沈澱させて秤量して各アミノ酸含量とした。即ちヒスチヂンは diflavianate, アルギニンは monoflavianate, リジンはpicrateとして沈澱させた。他の細かい操作はBlockの著書を参照した[4]。実験順序を略述すれば、第1図になる。

以上の方法で各アミノ酸誘導体に各々の係数を乗じて遊離のアミノ酸に換算する。測定は季節による変化、新鮮肉及び乾燥肉による差異、塩基性窒素の分布状態をしらべた。

季節による含量変化は第1表の如くである。

Table 1. The Basic Amino Acid Content of Surume-ika Meat in Summer and Autumn.

	Surume-ika in Summer		Surume-ika in Autumn	
	% of dry Matter	$\frac{\text{Basic Amino-N}}{\text{Total-N}}\times 100$	% of dry Matter	$\frac{\text{Basic Amino-N}}{\text{Total-N}}\times 100$
Histidine	1.46	4.59	1.20	2.56
Arginine	7.53	30.58	5.80	16.73
Lysine	7.01	15.45	5.16	7.67

この結果に依れば、アルギニン、リジン、ヒスチヂンの順に乾物に含まれる量が減じている。季節による変化は夏イカの方が含有量が多い。その割合は秋イカは夏イカのヒスチヂン含量の82.2%，アルギニンでは77.0%，リジンでは73.6%である。これは夏イカは成長期にあるためと考えられる。

次に新鮮肉と乾燥肉との差違を見ると第2表の如くなる。乾燥肉の調製は普通のスルメ製造工程と同一条件で行い、試料としては秋イカを用いた。

含量の順序は変化がないが、乾燥肉は新鮮肉に比し多くなつている。増加の割合はヒスチヂンは6.9%，アルギニンは20.6%，リジンは13.1%で、アルギニンは最も増加の割合が大である。

次に夏イカにつき素乾したスルメと煮熟後乾燥せるものにつき塩基性アミノ酸含量を決定せるものは第3表の如くである。

含量の順序は変らないが、アルギニン、リジンは煮熟イカではスルメより多くなつている。ヒスチヂンは却つて減少している。

第4表は全窒素に対する塩基性アミノ酸の割合と各種塩基性アミノ酸の比率を示したものである。

Fig.1

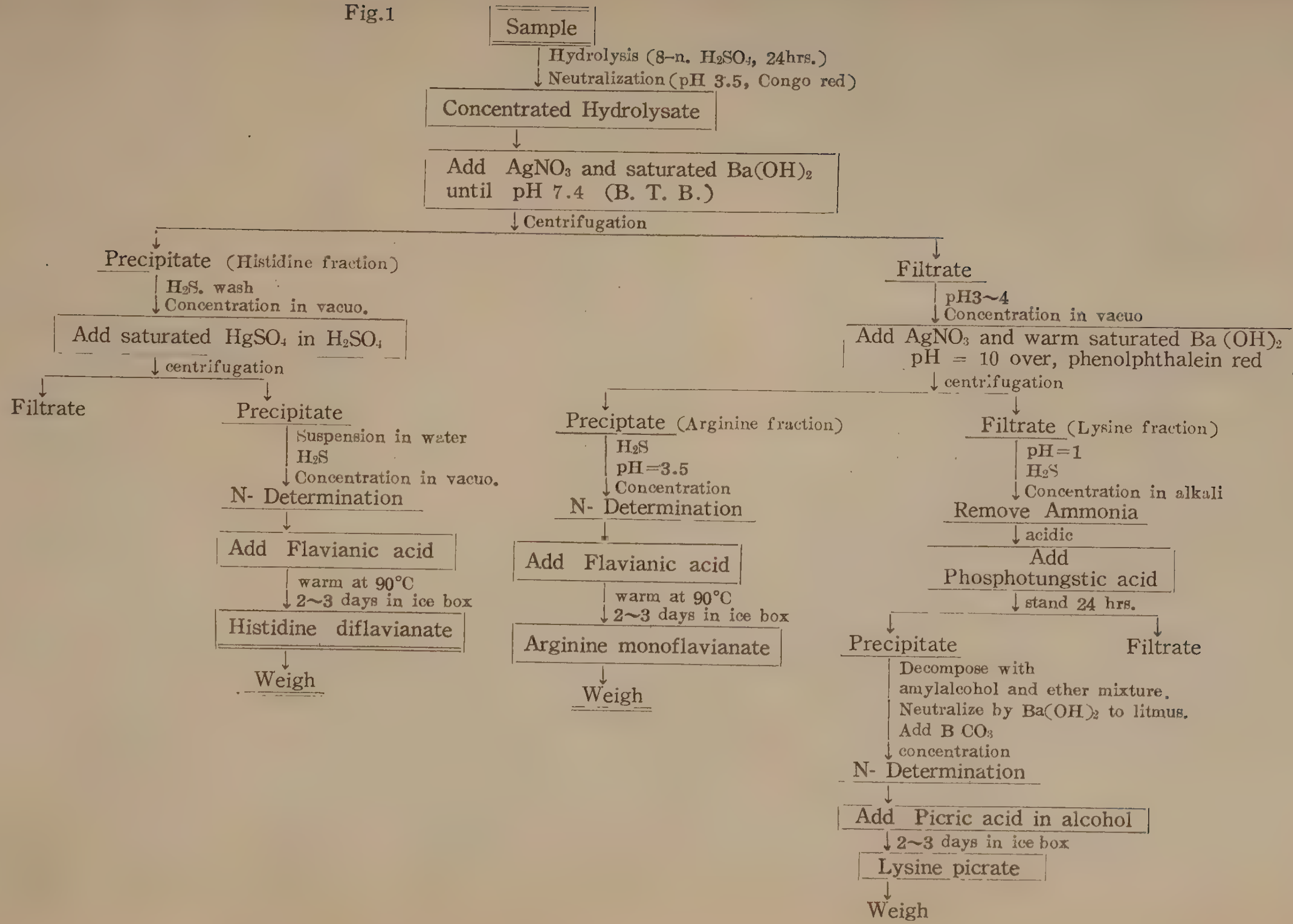

Sample
Hydrolysis (8-n. H_2SO_4, 24hrs.)
Neutralization (pH 3.5, Congo red)
Concentrated Hydrolysate
Add $AgNO_3$ and saturated $Ba(OH)_2$ until pH 7.4 (B. T. B.)
Centrifugation
Precipitate (Histidine fraction)
H_2S. wash
Concentration in vacuo.
Add saturated $HgSO_4$ in H_2SO_4
centrifugation
Filtrate
Precipitate
Suspension in water
H_2S
Concentration in vacuo.
N- Determination
Add Flavianic acid
warm at 90°C
2~3 days in ice box
Histidine diflavianate
Weigh
Filtrate
pH3~4
Concentration in vacuo
Add $AgNO_3$ and warm saturated $Ba(OH)_2$ pH = 10 over, phenolphthalein red
centrifugation
Preciptate (Arginine fraction)
H_2S
pH=3.5
Concentration
N- Determination
Add Flavianic acid
warm at 90°C
2~3 days in ice box
Arginine monoflavianate
Weigh
Filtrate (Lysine fraction)
pH=1
H_2S
Concentration in alkali
Remove Ammonia
acidic
Add Phosphotungstic acid
stand 24 hrs.
Precipitate
Filtrate
Decompose with amylalcohol and ether mixture.
Neutralize by $Ba(OH)_2$ to litmus.
Add B CO_3
concentration
N- Determination
Add Picric acid in alcohol
2~3 days in ice box
Lysine picrate
Weigh

Table 2. The Basic Amino Acid Content in Fresh Meat and Dry Meat of Surume-ika.

	Fresh Meat (Surume-ika in Autumn)		Dry Meat (Surume-ika in Autumn)	
	% of dry Matter	Basic Amino-N / Total-N ×100	% of dry Matter	Basic Amino-N / Total-N ×100
Histidine	1.19	1.82	1.20	2.56
Arginine	4.81	9.59	5.80	16.73
Lysine	4.56	4.90	5.16	7.67

Table 3. The Basic Amino Acid Content of Surume-ika Meat treated by Drying and Boiling.

	Surume (dry Meat, Surume-ika in Summer)		Boiled Meat (Surume-ika in Summer)	
	% of dry Matter	Basic Amino-N / Total-N ×100	% of dry Matter	Basic Amino-N / Total-N ×100
Histidine	1.46	4.59	1.11	3.74
Arginine	7.53	30.58	10.99	32.38
Lysine	7.01	15.45	9.60	21.73

Table 4. The Distribution of Basic Amino Acid Nitrogen.

	Total Basic Amino-N / Total-N	Against Total Basic Amino-N		
		Histidine-N	Arginine-N	Lysine-N
	%	%	%	%
Fresh Meat of Surume-ika in Autumn	16.35	11.14	58.65	30.25
Surume (Dry Meat) of Surume-ika in Autumn	26.91	9.51	61.97	28.50
Surume (Dry Meat) of Surume-ika in Summer	45.62	10.08	56.05	33.87
Boiled Meat of Surume-ika in Summer	57.87	6.48	55.97	37.56

全窒素に対する塩基性窒素は夏イカは秋イカに比して非常に多い。塩基性窒素中アルギニンの占める割合は非常に多い。

考　　察

以上栄養上重要な意義を有する塩基性アミノ酸の含有量を定量せる結果をみるに、アルギニン、リジン、ヒスチヂン共に夏イカは秋イカより大であつて、奥田氏、大谷氏、山川氏等が各種魚類のアミノ酸を分離定量せる数字と比較するも決して一般魚類に劣るものではない。

又Block の分析表を参照するもその全量は大体鶏の卵黄蛋白質なるビテリンに似ている。勿論栄養上必要欠くべからざるアミノ酸は以上3種のアミノ酸以外にスレオニン、メチオニン、トリプトファンその他多く存在するので之等につき定量すること、尚之等の消化吸收の難易も問題となるのであり

之等を明かにして初めて断定を下し得るのであり、尚最後に詳細な生物飼育試験によつて決定されねばならない。

要　　約

1. イカの栄養上の位置及び価値を定めんがためにこの研究を行つた。
2. 測定は塩基性アミノ酸について行い、Kossel改良法、Block法により、各アミノ酸の誘導体、即ち、ヒスチヂンヂイフラビヤナート、アルギニンモノフラビヤナート、とリジンピクラートとして分離定量した。
3. アミノ酸含量はアルギニン、リジン、ヒスチヂンの順に少くなる。
4. 季節的変化はすべて夏イカは秋イカよりも含量が多い。
5. 製品による変化は、新鮮肉は乾燥肉より含量は少いが、ヒスチヂンは変化がなかつた。
6. スルメと煮熟イカを比べたとき、煮熟イカはリジンが特に多く、ヒスチヂンは却つて減少している。
7. 塩基性窒素は夏イカに多く、特にアルギニンの占める割合が多い。
8. 以上の結果から塩基性アミノ酸より比較して、他の魚類とさして大差はない。
9. 決定的な栄養価値は今後その他の不可欠アミノ酸の存在量の確認と生物飼育試験によらねばならぬ。

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（水産科学研究所業績　第126号）

海温用抵抗温度計の精度について

小　藤　英　登（海洋学教室）

ON THE ACCURACY OF THE RESISTANCE THERMOMETER USED FOR MEASURING SEA WATER TEMPERATURE.

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The resistance thermometer inevitably contains errors caused by the Joule's heat generated in the temperature measuring bulb, and by the variations in the resistance of the lead wires due to the variations in temperature. But, when thermometer is used in ths sea water, the former of these errors decreases as the water current velocity increases, and tends to attain a constant value with increasing water velocity.

The author designed a resistance thermometer, and measured its accuracy and time constant.

In the present paper, he reports that the resistance thermometer designed by him can be used to measure the temperature with an accuracy of 0.01°C, the sensibility 10μA/°C in the sea water running at an ordinary velocity, though its time constant 13.5 sec. is not a small value.

1. 緒　　言

電気抵抗温度計は夙くから海洋測器として使われたが、その精度についての充分な検討は行われなかつたようである。これを裸線として気温の測定に使用する場合については、さきに渡辺氏による自熱誤差及び time lag の報文[1]がある。しかし水温の測定に使用する際は、感温部を水密性にする必要から構造が複雑となるために、その性能も著しく変つて来る。感温部の構造には種々の方法はあるが、以下筆者試作使用のものについてその精度を検討した結果を報告する。

2. 予備的考察

感温部を流れる電流によるJoule's heatの影響を自熱誤差と呼ぶこと、及びその表示を以下渡辺氏に踏襲する。

ブリッヂの検流計で読み取られる電流値は、感温部の外囲の温度に比例する量と自熱誤差による量との和

$I = i_G + \triangle i_G$……(1) である。

感温部の温度と外温との差θの時間的変化は

$\frac{d\theta}{dt} = \frac{1}{C}(dQ - dq)$　但し$dQ = \frac{i^2R}{4.185}$（單位時間の発熱量）　$dq = f(k,v)\theta$（單位時間の放熱量）

と表わされる。こゝにi,Rは感温部を流れる電流及びその抵抗値、C, k は夫々感温部の構造による常

数、vは外囲の流速である。

$t=0$ で $\theta=0$ のとき上式の解は

$$\theta=\theta_{0}\ (1-e^{t/\lambda})\quad 但し\quad \theta_{0}=\frac{i^2R}{4.185f(k,v)}=B(k,v)i^2,\quad \lambda=\frac{C}{f(k.v)}$$

である。このθ_0が温度としての自熱誤差量である。

測定流体の温度をTとすれば、温度計としての感度は$\frac{di_G}{dT}=Ai$（Aはブリッヂの回路について一定）であるから、(1)式は

$I=ATi+ABi^3$ ……(2) と表わされる。

温度計の感度を高めるためにはiの値を大きくしなければならないが、そうするとそれに伴つて自熱誤差量が著しく大きくなる。しかし自熱誤差量が大きくとも、感温部の一つの構造について流速の実際的範囲内でその変化が小さければ、$B(k,v)i^2=const.$ としてその値を含めたまゝ calibrate すれば感度を高くしても差支えないわけである。

上式中λは time lag を表わすものではあるが、Bの値から f (k,v) の値が定つても、Cの値が決らなければλは決らない。Cは感温部の簡単な構造ではその熱容量であるが、構造が複雑なときは感温部内の温度分布が外部の流速によつて変化し、即ち流速の函数と考えなければならないので、λの値は別に実験的に決めなければならない。

3. 実験方法及び結果

i. 自熱誤差の測定

測定装置： Fig. 1は筆者使用の抵抗温度計回路図である。150μAの検流計を使用し、感度を10μA/°Cとするために測温範囲を（0～15°C)、(10～25°C）の2段切換えにしてある。R_2を構成する抵抗

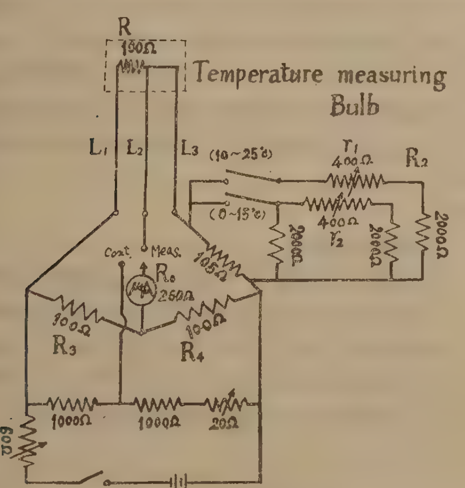


Fig 1. Temperature measuring Circuit.

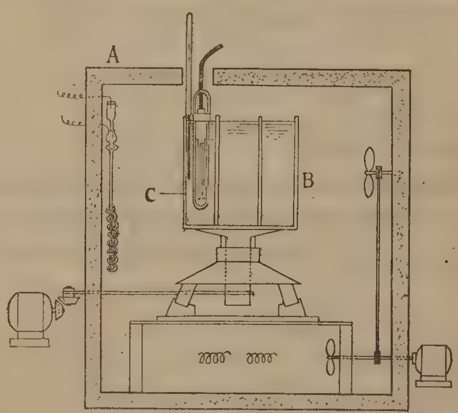


Fig. 2. Diagram of Apparatus.
A : Themost
B : Rotating Water Vessel
C : Temperture measuring Bulb.

中のr_1, r_2によつて検流計の目盛を温度の読みと一致させ得る。回路中 1,000Ω, 2,000Ω の高抵抗を使つたのはスキッチの抵抗変化の影響を避けるためである。感温部は0.08mm の銅線を 1.5mmのアルミニユーム管を絶縁したもの4本の上に巻き、夫々を内径3mm の銅管4本に直列に挿入してこ

れを水密にし、Fig.2に見られるような形状のものとした。

Fig.2 は恒温水流を得るための実験装置図である。恒温水流を得るために、恒温箱内にて円壔形水槽を廻転台の上に置き、これを外部からモーターによつて廻転させる。こうすることによつて大体定常的な層流を得ることが出来た。水槽を二重にしたのは、水槽の中心に近づくほど流速が著しく減ずるのを防ぐためである。流速は二重の水槽の中央部に2本の硝子管を挿入して動圧、静圧の差を以て測り、この速度を感温部の位置の流速とした。

測定及び測定結果： 恒温箱内の水槽中の温度Tが上下に於ても温度差なく、且つ一定流速になつたことを確めた後(2)式のIを測定する。Tの測定にはベックマン寒暖計を用い、測定の前後2分間の温度変化を $^5/_{100}$°C 以下に保つ。感温部を流れる電流 i はFig.1の導線L_1中に検流計を挿入して測定した。

流速を一定に保ち、電源電圧を変えて i を変化させ、数個の i の値に対してIを読みとり、夫々の I, T, i を(2)式に入れてA及びAB、従つてA及びBを決定する。次にT, i の同じ条件で流速を種々に変化させて夫々の流速に対するA及びBを決定する。それらの測定はTが18.33°Cから18.35°C の範囲内で行つた。

得られた流速vに対するA, Bの値をTable 1に、Bと流速vとの関係を Fig.3 に示す。Bの値は流速の増加とともに減少し、流速8.0cm/sec以上では一定の値になつてしまう。Fig.3 の曲線を

$$B\times10^{-3}=0.39e^{-0.32v}+1.09 \cdots\cdots(3)$$

と表し、これから得られるBの値による自熱誤差 Bi^2と流速vとの関係を感度10μA/°C から2μA/°C まで5種の値について図示すると Fig.4 のようになる。即ち自熱誤差は感度が低いほど小さく、且つ感度が低いほど流速の小さい値で一定値に近づく。それでいま温度計を calibrate する際、自熱誤差がこの極限値を示す充分速い流速のもとで行つたとして、それを基準に$^1/_{100}$°Cの精度を許容し得る流速の下限を知ろうとすれば、Fig.4 からもわかるが、(3)式から

Table 1. Values of A and B in eq. (1) for various Water Velocities.

v cm/sec.	$A\times10^5$	$B\times10^{-3}$
1.3	75	1.30
4.3	〃	1.25
8.0	〃	1.09
10.0	〃	〃
16.9	〃	〃

$$(B-1.09\times10^3)\,i^2=0.39e^{-0.32v}\times10^3 i^2<0.005$$

として、上記夫々の感度について vの値を求めると、次表のようになる。

di_G/dT	10μA/°C	8	6	4	2
v	7.9cm/sec	7.1	5.0	2.4	0

実際の使用に際しては、波による船の動揺及び海潮流による感温部と海水との相対速度が大体常に 8cm/sec. 以上にあることを考慮すれば、このような温度計を感度10μA/°C として$^1/_{100}$°C の精度にて測度することは充分に許容し得るものとして差支えないことがわかる。

ii. Time lag の測定

測定装置： Time lag の測定には自熱誤差の測定に使用したFig. 2 の装置をそのまゝ使用した。

先づ恒温箱内の水槽の温度を一定に保つて廻転させる（但し温度計の感温部は恒温箱外にある)。一定速度の層流を得たとき、恒温箱外で水温より約4°Cの低温に保つた感温部を恒温箱の上部にあけた小穴から急速に落下させて水槽内の水流中央に置く。この時から5sec. 毎に検流計の指度を読みとつ

て、普通の方法(2)で time constant を決定する。

測定及び測定結果： 温度計の感度が$10\mu A/^\circ C$で測温の精度が $1/100^\circ C$ を許容し得る水流の流速範囲で12cm/sec. の流速を選んだ。水槽の始めの温度は18.06°C、感温部の始めの温度は13.98°C、感温部の挿入とともに水槽内の流速も温度も急速に変化して約20sec. を経た後一定状態を示し、水温は18.04°C一定を維持した。12cm/sec. の流速は20sec. 以後の流速である。

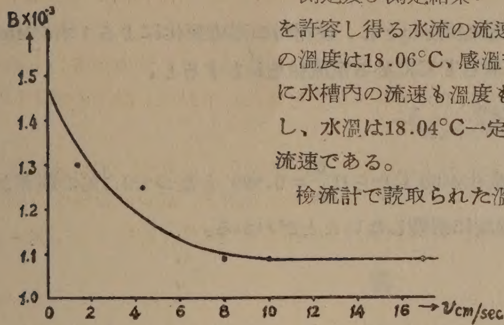


Fig 3. Relation between B in ep.(2) and Water Velocity

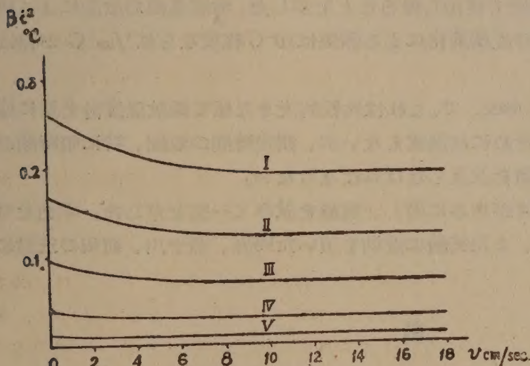


Fig 4. Temperature Error Curves for various Sensibilities I: $\frac{di_G}{dT}=10\mu A/^\circ C$, II: $8\mu A/^\circ C$, III: $6\mu A/^\circ C$ IV: $4\mu A/^\circ C$, V: $2\mu A/^\circ C$

検流計で読取られた温度の時間的変化をFig.5（実線部分）に示す。始めの数秒間は感温部の構造及び流速の変化等のために温度変化も異状を示すが、10 sec. 以後の温度上昇曲線は

$$\theta=\theta_\circ-(\theta_\circ-\theta i)\,e^{-0.074t}$$

と表わされる。但し$\theta_\circ$は外囲の温度、θiは感温部の始めの温度である。従つて time constant は$1/0.074=13.5$となる。上式の表す曲線は始めの数秒間はFig.5の点線で示された部分となるので、実際の指度の遅れは上述のtime constant で求められる遅れより数秒大きいものと考えれば充分である。但しこゝで指度の遅れは検流計の指度の遅れを含んでゐること勿論である。

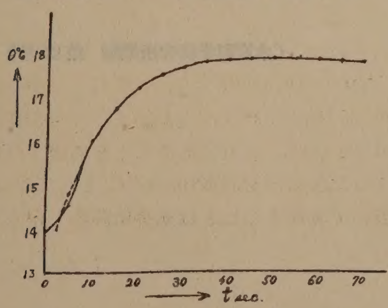


Fig 5. Rise of Temperature of Temperature measuring Bulb.

4. 感度及び精度に影響する諸種の検討

i. 温度による感度の変化

2に於て、前記渡辺氏の表示を踏襲して感度を$\frac{di_G}{dT}=Ai$と表し、感度は単に感温部を流れる電流のみによるものとして一定温度に於てAの値を決定したが、厳密には

$$\frac{di_G}{dT}=\frac{(Ai)_\circ}{1+\alpha T}$$ 但しα: 測温部抵抗の温度係数

であつて、感度は測温の温度によつて異るのである。但し$(Ai)_\circ$は0°C に於る感度で且つ0°Cに於て$R=R_2$ 即ち $i_G=0$ とする。

従つて上式より感度は温度が高い程減少して、T=15°Cでは 0°C の94%に、30°Cでは88%と低下する。

ii. Lead wire の温度変化による影響

抵抗温度計は感温部とブリッヂとの間のlead wireの温度変化に伴う抵抗変化による誤差を小さくするために、一般にFig. 1 のように3本のlead wire によつて連結する。しかし温度計を海洋測器として使用する場合は lead wire も長いもの

を使用するし、また船上に於る使用時の温度が調整検定時に於る温度と余程異るので、その部分の温度変化による誤差は多少とも免れ得ない。

いま調整検定時の感温部T°Cに於る検流計の電流値をi_Gとし、使用時の温度変化による1本のlead wireの抵抗変化を$_\Delta R$としてそのときの感温部の同じT°Cに於る電流値をi_G'とすると、

$$\frac{i_G'}{i_G}=\frac{3_\Delta R}{2R_0+R_2+R_4+3_\Delta R}\left(1+\frac{\alpha T_\Delta R}{R_2(1+\alpha T)+_\Delta R}\right)$$

で1本のlead wireの抵抗を2Ωとすると、温度変化が30°Cならば$\frac{i_G'}{i_G}=0.999$となつて0.1%の誤差が表われるが、10°C程度ならば1.000で$^1/_{100}$°Cの精度に影響しないことがわかる。

5. 結　　　言

抵抗温度計の許容精度を検討するため、筆者試作の温度計について自熱誤差を外囲流速の函数として測定した。その結果感度を10μA/°Cに高めても自熱誤差は流速8cm/sec. 以上では一定となるのでその流速範囲内でcalibrateすれば$^1/_{100}$°Cの精度で使用し得ることを示した。尚感温部の温度による感度の変化は30°Cで0°Cのときの12%減、導線の温度変化による誤差は10°C程度ならば$^1/_{100}$°Cの精度に影響しないことを検討した。

Time lag 測定の結果time constant は13.5sec. で、これは比較的大きな値で抵抗温度計を単に遠隔測器として船上で読み取り得るという便のためには差支えないが、測定時間の短縮、特に短時間の変化を読み取る等のためには更に測温部の構造を改良しなければならない。

近時この種の抵抗温度計が広く使われる向きがあるに際し、実験を試みて一文を草した。本文を草するに当り御指導御鞭韃を賜つた井上教授に、また実験に援助を頂いた内海、茄子川、前田の三君に厚く感謝の意を表する次第である。

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